Chemical Name: Afidopyropen
USEPA PC Code: 026200
USEPA MRID: 49689228
USEPA DP Barcode: 435146
PMRA Data Code: 9.2.4.9
PMRA Study No. (UKID): 2627498
Data Requirement (Guideline): OCSPP 850.3040 Guideline

Test Material: BAS 440 00 I (TEP, VERSYS™) **Purity:** 9.8%

Active Ingredient: Afidopyropen
IUPAC Name: [(3*S*,4*R*,4a*R*,6*S*,6a*S*,12*R*,12a*S*,12b*S*)-3-(cyclopropylcarbonyloxy)-1,2,3,4,4a,5,6,6a,12a,12b-decahydro-6,12-dihydroxy-4,6a,12b-trimethyl-11-oxo-9-(3-pyridyl)-11*H*,12*H*-benzo[*f*]pyrano[4,3-*b*]chromen-4-yl]methylcyclopropane carboxylate
CAS Name: [(3*S*,4*R*,4a*R*,6*S*,6a*S*,12*R*,12a*S*,12b*S*)-3-(cyclopropylcarbonyl)oxy)]-1,3,4,4a,5,6,6a,12,12a,12b-decahydro-6,12-dihydroxy-4,6a,12b-trimethyl-11-oxo-9-(3-pyridyl)-2*H*,11*H*-naphtho[2,1-*b*]pyrano[3,4-*e*]pyran-4-yl]methyl cyclopropanecarboxylate
CAS No.: 915972-17-7
Synonyms: INSCALIS™

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Biologist, USEPA/OCSPP/OPP/EFED/ERBIV
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Date: 15 February 2018

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Signature: THOMAS STEEGER
Date: 15 February 2018

PMRA Reviewer: Vedad Izadi
Evaluation Officer, PMRA/EAD/ERSII
Date: 16 October 2017

Date Evaluation Completed: 16 October 2017

CITATION: Schnurr A. 2015. Effects of BAS 440 00 I on the honeybee *Apis mellifera* L. under field conditions with additional assessments on colony and brood development. BioChem agrar Labor fur biologische und chemische Analytik GmbH, Gerichshain, Germany. Report No. 721403. Sponsor: BASF SE. Report No. BASF Reg. Doc. #: 2015/1001363. USEPA MRID 496892-28. PMRA UKID 2627498.

Executive Summary:
The full field study tested the effects of the afidopyropen formulated end-use product BAS 440 00 I (9.8% active ingredient) on honeybee (*Apis mellifera*) colonies with the intent of examining brood (*i.e.*, eggs, larvae, pupae) strength and colony strength (number and condition of adult bees/brood and available food reserves). The study design was based on the OCSPP 850.3040 guideline. Replicate bee

colonies (containing 15083 ± 555[1] adult bees/colony) were placed in phacelia (*Phacelia tanacetifolia*) fields, and while phacelia was in full bloom and bees were actively foraging, fields were treated with either 0.5 L/ha (50 g a.i./ha; 0.045 lbs a.i./A) of BAS 440 00 I, or a water (negative) control treatment. Each treatment group consisted of seven replicate bee colonies located in a single field. Colonies were placed in test plots for nine days (exposure phase), and then on the morning of the tenth day after treatment (10 DAT) colonies were moved to an alternate site for the remainder of the study (monitoring phase). Mortality and behavior were recorded daily from three days before, to 43 days after, treatments (-3 to 43 DAT). Assessments also included foraging activity (-3 to 9 DAT), colony condition (food stores, brood status) and strength (numbers of adults and pupae) at -1, 3, 10, 21, 31 and 43 DAT.

The preliminary brood check indicated healthy colonies with all brood stages present, and a sufficient supply with nectar and pollen. Throughout the study, the number of food or brood cells did not differ statistically among the two treatment groups. Treatment rates were not confirmed analytically and are therefore based on nominal treatment levels.

There were no statistically significant differences in adult worker bee mortality between the negative control and afidopyropen-treated colonies during the pre-application or monitoring phases of the study; during the exposure phase of the study, mean adult worker bee mortality was significantly ($p<0.05$) different (*i.e.*, 33% higher) in colonies treated with afidopyropen relative to the negative control colonies. Differences in worker bee mortality over the exposure phase was due largely to increases in mortality of 304 and 255% (compared to the control) late on 0 DAT after applications and on 1 DAT. No dead pupae were found in negative control or afidopyropen colonies during the pre-application, exposure and monitoring phases. There were no statistically significant ($p <0.05$) differences in bee foraging activity between afidopyropen-treated colonies and the negative control during the pre-application phase of the study; during the exposure phase of the study, relative to negative control colonies mean foraging activity was significantly ($p <0.05$) different (*i.e.*, 11% lower) in afidopyropen-treated colonies. Differences in foraging activity over the exposure phase was due largely to reductions in foraging activity of 25% (compared to the control) late on 0 DAT after applications were made, though through 3 DATs foraging activity was still reduced by 7-10%.

Sublethal behavioral effects after afidopyropen-treatment on the day of application were noted, wherein approximately 200 bees were reported as falling from flowers during foraging or inactivity; however, no additional behavioral effects in honeybees in the afidopyropen colonies were noted for the remainder of the assessment period (i.e., 1-43 DATs).

There were no statistically significant ($p <0.05$) differences in mean colony weight between afidopyropen-treated colonies and the negative control at either the beginning of the study (*i.e.*, -1 DAT) or the conclusion of the study (*i.e.*, 43 DAT). At -1 DAT, afidopyropen-treated colonies were on average 6.3% heavier than negative control colonies, and at 43 DAT, afidopyropen-treated colonies were on average 3.9% heavier than negative control colonies. The mean number of adult worker bees in afidopyropen-treated colonies was comparable to that in negative control colonies throughout the study except for 10 DATs. On this assessment day, the mean number of worker bees in afidopyropen-treated colonies was significantly ($p<0.05$) different (*i.e.*, 11% higher, respectively) than the mean number of worker bees in the negative control colonies. The mean number of brood (eggs, larvae and

[1] Note that all means in this summary are followed by ± one standard error (SE).

pupae) and food (nectar and pollen) cells in afidopyropen-treated colonies was comparable to that in negative control colonies throughout the study. The amount of brood increased slightly over time, with on average (across treatments) a 7% increase in brood area through the two brood cycles encapsulated by the study. Likewise, the amount of food increased over time, with on average (across treatments) a 65% increase in food area over the 43-day study period.

Results Synopsis:
The study is generally consistent with OCSPP Guideline 850.3040, although there are some potentially important study deficiencies. Treatment levels were not analytically verified in the study, and due to possible effects of weather prior to and immediately following applications, there is some uncertainty regarding actual afidopyropen exposure levels. However, residue data provide some evidence that bees were exposed to afidopyropen in the afidopyropen treatment group.

Honey bee colonies treated with formulated afidopyropen at 50 g a.i./ha (0.04 lbs a.i./A) during active bee flight exhibited significant ($p<0.05$) adverse effects on adult worker bee mortality (*i.e.*, 33% higher) and foraging activity (*i.e.*, 11% lower), resulting in a no-observed adverse effect level (NOAEL) of <50 g a.i./ha under the conditions tested. Adverse effects on worker bee mortality and foraging activity occurred primarily on the day of applications (0 DAT) and on 1 DAT, and by the conclusion of the 43-day study afidopyropen-treated colonies were by all measures similar to or exceeded performance of the negative control colonies.

EPA Classification: Supplemental (should only be used qualitatively)
PMRA Classification: Reliable with restrictions

I. DATA SOURCE

USEPA MRID No.:	49689228
PMRA UKID No.:	2627498
Study Title:	Effects of BAS 440 00 I on the honeybee *Apis mellifera* L. under full field conditions with additional assessments on colony and brood development.
Study Author(s):	Schnurr A.
Testing Laboratory:	BioChem agrar Labor fur biologische und chemische Analytik GmbH, Gerichshain, Germany.
Laboratory Report No.:	721403
Sponsor Study No.:	BASF Reg. Doc. #: 2015/1001363
Study Completion Date:	1 December 2015
Data Access:	Data submitter is data owner
Data Protection Claimed:	Yes

II. MATERIALS AND METHODS

Test Guideline:	USEPA OCSPP 850.3040 Guideline: Field Testing for Guidelines (2012); also EPPO Standard PP 1/170(4) (2010)
Deviations from Guideline:	The treatment unit – and therefore appropriate sampling unit - in this field study is the plot itself, rather than the colonies within the plot; therefore, as designed this study does not contain statistically independent replicates. Despite the presence of pseudo-replication in

	this study, data were analyzed as if the colonies were independent (*i.e.*, n = 7 for both the negative control and afidopyropen treatment groups) using linear models, followed by *post-hoc* means comparison tests depending on whether data met assumptions for parametric statistical tests.
GLP Compliance:	Yes; signed GLP certificate was included and reported no guideline deviations. Laboratory certified by the Staatsministerium fur Umwelt und Landwirtschaft, Freistaat Sachsen.

A. MATERIALS

Test Material:	BAS 440 00 I (VERSYS™)
Test Material Identity	Batch No. FD-130925-0022; a yellow, liquid formulation comprising afidopyropen (BAS 440 I): 100 g/L (nominal), 98.2 g/L (9.8% measured).
Details on Preparation and Application of Test Materials:	All substances were applied in 400 L/ha water using a calibrated, portable plot sprayer. Applications were made to fully flowering phacelia.
Analytical Monitoring:	None
Details on Analytical Monitoring:	N/A
Reference material:	None
Vehicle:	None
Test Organism (Species):	*Apis mellifera* L. (honeybee)
Animal Group:	Arthropoda/Insecta/Hymenoptera/Apidae
Details on Test Organisms:	Healthy honeybee colonies with two bodies (hive boxes) containing eleven combs (overall 22-sides of comb) each, including 5-10 brood combs with all brood stages present, and 10-19 combs with food, were used for the study. At the first brood assessment, one day prior to treatment (-1 DAT), colonies contained 12,600-19,350 adult bees. Bees in the colonies were free of clear visual signs of disease or pests, and no unusual occurrences were reported in colonies prior to treatments. Sister queens from 2014 were used to produce colonies which were as uniform as possible (source: BioChem agrar GmBH, Gerichshain, Germany).

B. STUDY DESIGN AND METHODS

Study Type:	Full-field study
Test Duration Type:	Long-term toxicity test
Limit Test:	Yes
Total Exposure Duration:	9 d
Post-Exposure Observation Phase:	34 d for all endpoints
Remarks:	Bee mortality and behavior were assessed daily beginning three days before (-3 DAT) and ending at 34 DAT. Foraging activity of the bees was

assessed -3 to 9 DAT. Overall condition of the colonies (food stores, brood status and colony strength) were assessed-1, 3, 10, 15, 21, 31 and 43 DAT.

Colony strength and condition assessments were conducted according to the assumption that the maximum number of bees per colony consisting of two supers with a total of 22 comb sides and four bounding hive walls could theoretically be 43,200 bees (*i.e.*, 39,600 bees on combs, and 3,600 bees on walls). For assessments of colony strength it was further assumed that each comb side was separated into 8 equal sections covered by a theoretical maximum number of 900 bees/section, assessments were conducted by counting the number of "eights" covered by bees (assuming that each eight held 112.5 bees), and then extrapolating the number of "eights" per comb to the estimated total number of bees per colony. For assessments of colony condition and brood strength, it was further assumed that each comb side (825.1 cm^2 in area), and so each "eight" was 103.1 cm^2 in area; the final assessments of these endpoints was made relative to comb area per colony (cm^2/colony).

Residue analysis was conducted on phacelia floral and leaf tissues that were collected from random locations in the two fields once before application on -1 DAT, and once immediately after application (within 4 hours) on 0 DAT. An additional honeybee colony (*i.e.*, no. 8) was also located at each of the two fields, and used solely for the collection of residues from pollen and nectar samples. Nectar samples were collected from foraging bees by closing off the entrance hole of colony no. 8 for several minutes during active bee flight; returning forager bees circling the hive were collected using a sweep net and immediately frozen using liquid nitrogen for residue analysis. Pollen samples were collected from forager bees via pollen traps attached to the front of colony no. 8 on the day of sampling when bees were actively foraging; pollen traps were installed for roughly 4 hours, were then removed, and the purple phacelia pollen collected. Nectar and pollen were sampled once before application on -1 DAT, and then once on 1 DAT.

Test Environmental Conditions:

Ambient environmental conditions during the study were: 11.0-27.8 °C and 63-80% relative humidity (RH) before application; 24.1-24.3 °C and 26.8-27.1% RH during applications; 11.2-37.7°C and 52-66% RH during the 9-d exposure phase; and, 8.2-37.1°C and 47-92% RH between 10 and 43 DATs (*i.e.*, the monitoring phase of the study). Rainfall (>1.0 mm) was reported during the study at -3, 6, 8, 9, 12, 13, 14, 19, 21, 22, 23, 27, 28, 29, and 35 DAT and consisted of 4-5, 10-14, 7-9, 3, 1.6, 2.4, 2.0, 10.0, 1.6, 36.9, 6.0, 4.6, 3.7, 2.4, and 3.9 mm, respectively.

Photoperiod and Lighting: Natural

Nominal and Measured Concentrations:

Negative control: tap water (400 L/ha)
Test item: afidopyropen: 0.5 L/ha (50 g a.i./ha [nominal])

Test Plots: The negative control test site was 2.2 ha in area and located in 04808 Altenbach, Saxony, Germany, and the afidopyropen treatment site was 2.1 ha in area and located at 04319 Althen, Saxony, Germany. Fields selected for the study had not been used for agricultural production during the past five years, and were converted to phacelia crops for the purpose of the study. No other pesticides were applied at the test plots for at least three years prior to sowing of phacelia for the study. Fields were located in an area where no other phacelia fields or other bee-attractive crops were nearby.

Test Design: Full-field test, with seven bee colonies per 2.1-2.2 ha treated test plot. Healthy bee colonies were introduced on the evening of 26 June 2015, four days before application (-4 DAT). The application was carried out four days later during bee flight at full flowering of the crop (BBCH 65, full flowering).

Bees were exposed to the water and afidopyropen-treated phacelia at test plots for nine days. At 10 DAT, colonies were removed from the test plots and relocated to a monitoring site approximately 6.7 km away (04821 Brandis, Saxony, Germany), which was located in an area without flowering crops or intensive agriculture (study author's characterization).

III. APPLICANT'S REPORTED RESULTS AND DISCUSSION

Exposure Duration: 9 d
Endpoint(s): Mortality, foraging activity, behavior, colony development (*i.e.*, colony strength, colony weight, and brood development)
Effect Concentration: ≥ 0.5 L/ha
Basis for Concentration: Nominal
Effect Concentration Type: Test material
Basis for Effect: Adult mortality, foraging activity, sublethal behavioral effects, colony strength and condition, residues.

Applicant-Provided Results:

Application Conditions & Deviations: Applications were made using a tractor-mounted sprayer (Amazone UG 2200 Special, Bodenbearbeitungsgerate Leipzig GmbH & Co KG, Germany) roughly 0.5 m above the crop canopy at an application speed of 6.5 km/h. Applications to the negative control test plot were made at 9:15-9:35 AM, and those to the test item plot were made at 10:23-10:43 AM, on 30 June 2015. Mean bee foraging activity prior to applications in the control plot was reported to be 9.4 bees/m^2, and mean bee foraging activity prior to applications in the afidopyropen plot was reported to be 10.2 bees/m^2. At the time of applications, wind speed for all applications was 0.7-0.9 m/s^2, temperatures were 21.9-24.3 °C, and relative humidity was 56.8-64.3%. The amount of applied product

(based on application volumes) deviated from the target application amount by +6.0% for afidopyropen applications.

Sublethal Behavioral Effects: According to the study author, no abnormal honeybee behavior was reported in either the negative control or afidopyropen colonies prior to applications. On the day of applications (0 DAT), immediately following applications, the study author reported that "several" bees reportedly "fell" from phacelia flowers while foraging. Two hours after applications, again in afidopyropen colonies, 20, 12, 38, and 70 bees, respectively, in the dead zone dead bee traps of colonies 1, 2, 6 and 7, were reported to be "inactive." In the evening after applications, amongst afidopyropen colonies, 15, 10, 5, 28 and 200 bees, respectively, in the dead zone dead bee traps of colonies 1, 2, 3, 6 and 7, were reported to be "inactive." No additional behavioral effects in honeybees in the afidopyropen colonies were noted following these reported incidences, *i.e.*, for 1-43 DATs.

Adult & Juvenile Mortality: According to the study author, there were no statistically significant differences in adult worker bee mortality between the negative control and afidopyropen-treated colonies during the pre-application phase of the study (see **Table 1**). During the exposure phase of the study, mean adult worker bee mortality was reportedly significantly ($p<0.05$) different (*i.e.*, higher) in colonies treated with afidopyropen relative to negative control colonies. Apparently, on the day of application following treatment (*i.e.*, 0aa DAT), adult worker bee mortality in colonies that received afidopyropen applications was significantly ($p <0.05$) higher (mean ± std dev 0aa DAT worker bee mortality = 14.0 ± 6.3 dead bees/colony/day) than in negative control colonies (mean 0aa DAT worker bee mortality = 4.6 ± 1.4 dead bees/colony/day). There were no statistically significant differences in adult worker bee mortality between the negative control and afidopyropen-treated colonies during the monitoring phase of the study.

According to the study author, during the pre-application, exposure and monitoring phases, no dead pupae were found in negative control or afidopyropen colonies; therefore, the study author did not perform statistical analyses on pupal mortality data (**Table 1**).

Table 1. Study author-reported effects on bee (*Apis mellifera*) mortality and foraging activity under full-field conditions at pre-application, exposure phase, and post-exposure monitoring phase for negative control and formulated afidopyropen-treated (BAS 440 00 I, 9.8% a.i.) colonies (means ± standard deviation are reported).

	Control	Afidopyropen
Mean mortality of adult worker bees (n dead bees/colony/day)		
Pre-application phase [1]	7.6 ± 4.4	8.7 ± 5.2
Exposure phase [1] (0 – 9 DAT)	6.0 ± 3.9	8.2 ± 9.0 *
Monitoring phase [2] (10 – 43 DAT)	2.9 ± 2.5	2.7 ± 2.6
Mean mortality of pupae (n dead pupae/colony/day) [3]		
Pre-application phase	0.0 ± 0.0	0.0 ± 0.0
Exposure phase (0 – 9 DAT)	0.0 ± 0.0	0.0 ± 0.0
Monitoring phase (10 – 43 DAT)	0.0 ± 0.0	0.0 ± 0.0
Mean foraging activity (bees/m^2/colony/day [n])		
Pre-application phase	7.8 ± 2.2	8.0 ± 2.4

Exposure phase (0 – 9 DAT)	10.0 ± 1.5	9.2 ± 1.8
Mean colony weight (kg)		
Pre-application phase (-1 DAT)	26.4 ± 2.8	27.9 ± 2.6
Monitoring phase (43 DAT)	29.7 ± 2.2	30.9 ± 2.0

1) Sum of dead individuals found in dead bee traps and on linen sheets in the plots.
2) Mean number of dead honeybees per day and colony found in dead zone dead bee traps, only.
3) Data on mean mortality of pupae were not statistically analyzed by the study author.
* = statistically significant differences ($p < 0.05$) compared to the control, Student's t-test or Welch's t-test
DAT = days after treatment

Foraging Activity: According to the study authors, there were no statistically significant differences in mean foraging activity between colonies from the negative control and the afidopyropen-treated colonies at any point in the study (see **Table 1**).

Colony Weight: According to the study authors, there were no statistically significant differences in mean honeybee colony weight between colonies from the negative control and the afidopyropen-treated colonies at any point in the study (see **Table 1**).

Colony Strength: The study author did not statistically analyze colony strength (estimated number of bees per colony) data, but nevertheless stated that there was no indication of significant ($p<0.05$) adverse effects from afidopyropen treatments on overall colony strength (see **Table 2**). Overall, in negative control colonies, after 43 days colony strength decreased by 5% relative to -1 DAT, while in afidopyropen-treated colonies the mean number of bees/colony decreased by 3%.

Table 2. Summary of colony strength (mean number of worker bees) in negative control and afidopyropen-treated colonies at specified days after treatment (DAT). Table reproduced from applicant-submitted study report.

Assessment day	Colony strength [bees/colony]					
	Control			Test item		
	Mean[1)]	± SD	%[2)]	Mean[1)]	± SD	%[2)]
DAT -1	15316	2215	-	14850	2074	-
DAT 3	14496	2262	-5	14496	3112	-2
DAT 10	13613	1047	-11	15059	1308	+1
DAT 21	13291	845	-13	14111	1370	-5
DAT 31	15332	922	0	15605	608	+5
DAT 43	14529	1154	-5	14400	1243	-3

DAT: day after treatment 1) mean of seven replicates 2) relative change in comparison to DAT -1 calculated from the respective mean values

Colony Condition: The study author did not statistically analyze data on brood strength (*i.e.*, estimated brood area occupied by eggs, larvae or pupae) or food stores (*i.e.*, estimated brood area occupied by nectar and pollen), but nevertheless stated that overall, the applications of afidopyropen did not result in any adverse effects on brood strength (**Table 3)** or food stores (**Table 4**). In negative control colonies, after 43 days the estimated mean area of the total brood increased by 9% relative to -1 DAT, while in afidopyropen-treated colonies the estimated mean area of the total brood increased by 7%. Also, in

negative control and afidopyropen colonies, after 43 days estimated mean area of food stores increased by an average of 64% relative to -1 DAT.

Residues: The study author reported that no afidopyropen or its transformation product M4401007 residues were detected in flower, leaf, nectar or pollen specimens collected at random locations in negative control or afidopyropen plots before applications were made; additionally, no residues were reportedly found in specimens collected in negative control plot following applications. Immediately following (<4 h) applications, residues of afidopyropen and M4401007 in phacelia flowers were 4.40 and 1.85 mg a.i./kg, respectively. Residues of afidopyropen and M4401007 in foliage were 4.76 and 2.39 mg a.i./kg, respectively. Afidopyropen and M4401007 residues in pollen were 0.02 and 0.03 mg a.i./kg, respectively; and, residues in nectar were below the limit of quantitation (*i.e.*, LOQ<0.01 mg a.i./kg).

Weather Data: Weather data reported by the study author are summarized in **Figure 1**, and include total daily precipitation (mm), daily mean temperature (°C), daily mean humidity (% RH), and cloud cover (%) for the study. The study author noted that during the pre-application phase of the study, mean daily temperatures were 18.1-20.1 °C and there was 4-5 mm of rainfall (and 60-70% cloud cover) 3 days before application. During the exposure phase of the study mean daily temperatures were 15.3-29.6 °C, and there was substantial rainfall at 6 (10-14 mm), 8 (7-9 mm) and 9 DATs (3 mm). During the monitoring phase of the study, mean daily temperatures were 14.3-28.5 °C, and there was 2.4, 2.0, 10.0, 36.9, 6.0, 4.6, 3.7, 2.4, and 3.9 mm of precipitation, respectively, 13, 14, 19, 22, 23, 27, 28, 29, and 39 DATs.

Overall, the study author concluded that applications of BAS 440 00 I during bee flight (*i.e*., during the daytime) resulted in some transient effects on worker bee mortality, but that there were no long-term effects on any of the evaluated colony parameters.

Applicant-Reported Statistics and Error Estimates
The applicant reported means and standard deviations for all endpoints; the following endpoints were statistically analyzed by the study author: adult worker bee mortality; foraging activity; and, colony weight. ToxRat Professional (ver. 3.1.0) were used for all of the study author's statistical analyses.

Data were apparently tested for the homogeneity of variances per the study author's descriptions of statistical methods in the study report, but it is not clear what test was used for the comparison of variances, and it's not stated whether the distributions of data were tested for normality. Pre-treatment data were statistically evaluated using a Tukey's Test, and post-treatment data were statistically evaluated using pairwise Student t-tests or Welch's t-test for comparisons versus the control. All pre-application comparisons were made using two-sided tests, and all post-application comparisons were made using one-sided tests.

Table 3. Summary of brood strength (estimated brood area per colony) in negative control and afidopyropen-treated colonies at specified days after treatment (DAT). Table reproduced from applicant-submitted study report.

Assessment day	Brood development [estimated mean area of bee brood/colony (cm^2/colony)]					
	Control			Test item		
	Mean[1)]	± SD	[%][2)]	Mean[1)]	± SD	[%][2)]
	Eggs					
DAT -1	1621	627	-	1856	674	-
DAT 3	1842	664	+14	1724	501	-7
DAT 10	1930	518	+19	2217	602	+19
DAT 21	1628	315	0	1827	629	-2
DAT 31	1658	544	+2	1871	608	+1
DAT 43	2409	316	+49	1989	351	+7
	Larvae					
DAT -1	2446	729	-	2210	352	-
DAT 3	2269	729	-7	2431	586	+10
DAT 10	2151	478	-12	2063	413	-7
DAT 21	1812	420	-26	2100	523	-5
DAT 31	1827	341	-25	2114	566	-4
DAT 43	2497	357	+2	2402	538	+9
	Pupae					
DAT -1	4582	1855	-	4553	1389	-
DAT 3	5275	1638	+15	5510	1519	+21
DAT 10	5687	1507	+24	6078	1261	+33
DAT 21	4951	922	+8	4825	1311	+6
DAT 31	4538	726	-1	4626	977	+2
DAT 43	4538	599	-1	4825	1066	+6
	Total brood in all stages					
DAT -1	8649	2458	-	8619	1909	-
DAT 3	9386	2797	+9	9665	2315	+12
DAT 10	9769	1824	+13	10358	1802	+20
DAT 21	8391	1260	-3	8752	1747	+2
DAT 31	8023	736	-7	8612	1447	0
DAT 43	9444	939	+9	9216	1669	+7

DAT: day after treatment

1) mean of seven replicates

2) relative change in comparison to DAT -1 calculated from the respective mean values

Table 4. Summary of food stores (nectar, honey and pollen) in negative control and afidopyropen-treated colonies at specified days after treatment (DAT). Table reproduced from applicant-submitted study report.

Assessment day	Brood development [estimated mean area of bee brood/colony (cm^2/colony)]					
	Control			Test item		
	Mean[1]	± SD	[%][2]	Mean[1]	± SD	[%][2]
	Nectar					
DAT -1	6350	1435	-	5923	3670	-
DAT 3	10756	2365	+69	11544	2474	+95
DAT 10	13305	1750	+110	12524	2493	+111
DAT 21	11934	2021	+88	10852	2103	+83
DAT 31	10712	2161	+69	10144	1690	+71
DAT 43	11227	2378	+77	10328	1727	+74
	Pollen					
DAT -1	2210	1352	-	1871	1041	-
DAT 3	2107	1478	-5	1621	805	-13
DAT 10	1658	1084	-25	1370	895	-27
DAT 21	2070	585	-6	1481	404	-21
DAT 31	1805	447	-18	1407	421	-25
DAT 43	2844	905	+29	2475	622	+32
	Nectar + Pollen					
DAT -1	8560	2647	-	7794	3950	-
DAT 3	12863	3490	+50	13165	2938	+69
DAT 10	14962	2576	+75	13894	3108	+78
DAT 21	14005	1739	+64	12332	2037	+58
DAT 31	12516	1972	+46	11551	1601	+48
DAT 43	14071	2959	+64	12804	1509	+64

DAT: day after treatment

[1] mean of seven replicates

[2] relative change in comparison to DAT -1 calculated from the respective mean values

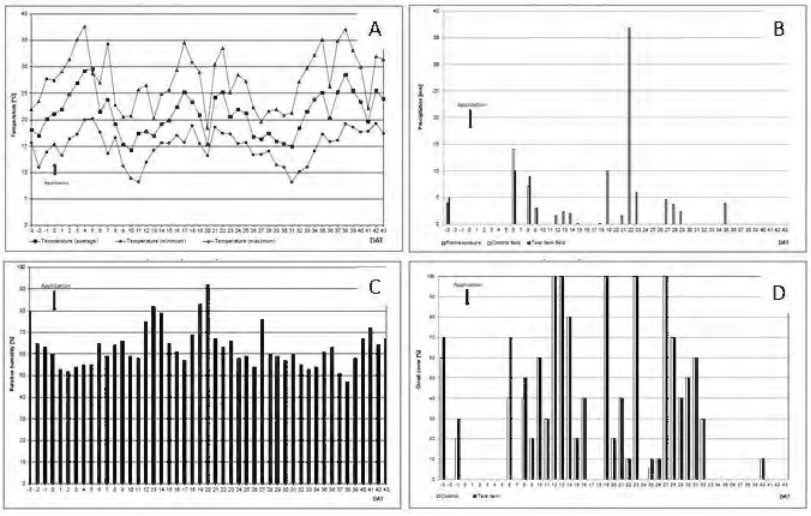

Figure 1. Summary of study author-provided data on daily temperature ('A'), precipitation ('B'), relative humidity ('C'), and cloud cover ('D'). Graphs reproduced from applicant-submitted study report.

IV. OVERALL REMARKS, ATTACHMENTS

Microsoft Excel data tables were submitted with an OECD-formatted summary by the registrant. The applicant did not include raw data on measured residues in the provided Excel tables, and so these data were manually extracted from the study report by the reviewer.

V. PRIMARY REVIEWER'S ANALYSIS AND CONCLUSIONS

The reviewer verified all of the applicant's calculations (where possible – see following note) and carried out statistical analyses per relevant EFED guidance for all data to confirm the applicant's results and conclusions.

Note that data on brood strength (mean number of eggs, larvae, and pupae), and food stores (mean number of cells as nectar or pollen), were expressed by the study author as colony area (*i.e.*, cm^2/colony). These colony area values were in turn estimated by the study author on the basis of the number of x/8 per frame side (number of "eights") counted as containing each type of brood or food cell, which is how the raw data on these variables were measured by the study author. The underlying assumption was that each comb/frame side (total area of 825.1 cm^2) consisted of 8 equal parts (each 103.1 cm^2) covered by brood, food, or empty cells; the total possible comb area per colony based on these assumptions was reported by the study author to be 18,152 cm^2. The study author further explained the assumption that each frame/comb side could by covered by a maximum of 900 bees, and so each "eighth" was assumed to contain 112.5 bees. So, data were expressed and analyzed (both by the study author and the reviewer) as estimated colony area (cm^2/colony) for brood strength (mean number of eggs, larvae, and pupae) and food stores (mean number of cells as nectar or pollen); data on the mean number of worker bees were expressed and analyzed (both by the study author and the reviewer) as the mean number of bees as estimated by extrapolation from the described area to colony population density assumptions.

Adult & Juvenile Mortality: There were no statistically significant differences in adult worker bee mortality between the negative control and afidopyropen-treated colonies during the pre-application phase of the study (see **Table 5**). During the exposure phase (0 – 9 DAT) of the study, mean adult worker bee mortality was significantly ($p<0.05$) different (*i.e.*, 33% higher) in colonies treated with afidopyropen relative to negative control colonies. There were no statistically significant differences in adult worker bee mortality between the negative control and afidopyropen-treated colonies during the monitoring phase (10 – 43 DAT) of the study. Differences in worker bee mortality over the exposure phase was due largely to increases in mortality of 304 and 255% (compared to the control) late on 0 DAT after applications and on 1 DAT.

No dead pupae were found in negative control or afidopyropen colonies during the pre-application, exposure and monitoring phases; therefore, the reviewer did not perform statistical analyses on pupal mortality data (**Table 5**).

Table 5. Reviewer-calculated effects on bee (*Apis mellifera*) mortality, foraging activity, and bee brood development under full field conditions at pre-application, exposure, and post-exposure monitoring

phases for negative control and formulated afidopyropen (BAS 440 00 I; 9.8% active ingredient)-treated colonies (means ± standard error are reported).

	Control	Afidopyropen
Mean mortality of adult worker bees (n dead bees/colony/day)		
Pre-application phase [1]	7.64 ± 0.83	8.68 ± 0.99
Exposure phase [1] (0 – 9 DAT)	5.42 ± 0.46	7.23 ± 0.85 †
Monitoring phase [2] (10 – 43 DAT)	2.87 ± 0.16	2.71 ± 0.17
Mean mortality of pupae (n dead pupae/colony/day) [3]		
Pre-application phase	0.0 ± 0.0	0.0 ± 0.0
Exposure phase (0 – 9 DAT)	0.0 ± 0.0	0.0 ± 0.0
Monitoring phase (10 – 43 DAT)	0.0 ± 0.0	0.0 ± 0.0
Mean foraging activity (bees/m^2/colony/day [n])		
Pre-application phase	7.80 ± 0.48	7.95 ± 0.51
Exposure phase	10.15 ± 0.18	9.04 ± 0.23 †
Mean colony weight (kg)		
Pre-application phase (-1 DAT)	26.41 ± 1.06	28.07 ± 0.91
Monitoring phase (43 DAT)	29.71 ± 0.81	30.86 ± 0.77

1) Sum of dead individuals found in dead bee traps and on linen sheets in the plots.
2) Mean number of dead honeybees per day and colony found in dead zone dead bee traps, only.
3) Data on mean mortality of pupae were not statistically analyzed by the study author.
† = statistically significant differences ($p < 0.05$) compared to the control, Wilcoxon rank sum test
DAT = days after treatment

Foraging Activity: There were no statistically significant ($p < 0.05$) differences in foraging activity between afidopyropen-treated colonies and the negative control during the pre-application phase of the study (**Table 5**). During the exposure phase of the study, relative to negative control colonies mean foraging activity was significantly ($p < 0.05$) different (*i.e.*, 11% lower) in afidopyropen-treated colonies. Differences in foraging activity over the exposure phase was due largely to reductions in foraging activity of 25% (compared to the control) late on 0 DAT after applications were made, though through 3 DATs foraging activity was still reduced by 7-10%.

Colony Weight: There were no statistically significant ($p < 0.05$) differences in mean colony weight between afidopyropen-treated colonies and the negative control at either the beginning of the study (*i.e.*, -1 DAT) or the conclusion of the study (*i.e.*, 43 DAT) (**Table 5**). At -1 DAT, afidopyropen-treated colonies were on average 6.3% heavier than negative control colonies, and at 43 DAT, afidopyropen-treated colonies were on average 3.9% heavier than negative control colonies.

Colony Strength: The mean number of adult worker bees in afidopyropen-treated colonies was comparable to that in negative control colonies throughout the study except for 10 DATs (**Table 6**). On this assessment day, the mean number of worker bees in afidopyropen-treated colonies was significantly ($p<0.05$) different (*i.e.*, 11% higher, respectively) than the mean number of worker bees in the negative control colonies.

Colony Condition: The mean number of brood (*i.e.*, eggs, larvae and pupae) and food (*i.e.*, nectar and pollen) cells in afidopyropen-treated colonies were comparable to that in negative control colonies throughout the study (**Table 6**). The amount of brood increased slightly over time, with on average (across treatments) a 7% increase in brood area through the two brood cycles encapsulated by the study. Likewise, the amount of food increased over time, with on average (across treatments) a 65% increase in food area over the 43-day study period.

Table 6. Reviewer-calculated effects on honey bee (*Apis mellifera*) colony strength and condition under full field conditions by day after treatment (DAT) for negative control and formulated afidopyropen (BAS 440 00 I; 9.8% active ingredient)-treated colonies (means ± standard error are reported).

Days after Treatment (DAT)						
Treatment	**-1**	**3**	**10**	**21**	**31**	**43**
Colony Strength – Adults (est. n adult bees/colony/d)						
Control	15316 ± 837	14497 ± 855	13613 ± 396	13291 ± 320	15332 ± 349	14529 ± 436
Afidopyropen	14850 ± 784	14497 ± 1176	15059 ± 494 †	14111 ± 518	15606 ± 230	14400 ± 470
Colony Condition – Brood (est. cm^2/colony as eggs, larvae or pupae)						
Control	2941 ± 369	3127 ± 411	3255 ± 433	2796 ± 364	2673 ± 317	3147 ± 238
Afidopyropen	2872 ± 328	3221 ± 420	3451 ± 450	2916 ± 354	2870 ± 318	3071 ± 317
Colony Condition – Food (est. n cm^2/colony as nectar or pollen)						
Control	4279 ± 676	6429 ± 1301	7478 ± 1657	7000 ± 1420	6256 ± 1298	7033 ± 1251
Afidopyropen	3896 ± 892	6580 ± 1454	6945 ± 1619	6164 ± 1356	5774 ± 1252	6444 ± 1150

† = statistically significant differences ($p < 0.05$) compared to the control, Wilcoxon rank sum test

Residues: Note that for analysis of afidopyropen residues in relevant matrices (*i.e.*, flowers, leaves, nectar and pollen) a single pooled sample was collected from the separate residue sampling-only afidopyropen plot, so no statistical analyses could be carried out on reported residue results for these data. Please reference Section III above for the study author's reported residue results.

Reviewer's Statistical Verification:
Statistical analyses confirmed using R (ver. 3.2.5)[2] statistical software, and the multcomp[3] analysis package. The reviewer relied on the Shapiro-Wilk's test and Bartlett's test to evaluate whether data were normally distributed or homoscedastic, respectively. ANOVA and Dunnett's multiple means test was used to test for statistical differences amongst means for data that met assumptions for parametric tests (*i.e.,* data were approximately normally distributed and had homogenous variances), and Kruskal-Wallis and Wilcoxon Rank Sum tests were used for non-parametric means comparisons. One-sided tests were used for all hypothesis-based testing; $\alpha = 0.05$ for all mean comparison tests, and $\alpha = 0.01$ for all assumptions testing.

See **Appendix I** for summary statistics and diagnostic tests (*i.e.,* goodness-of-fit and equivalent variances tests) for all data described in this data evaluation record.

[2] R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/.
[3] Hothorn T, F Bretz and P Westfall. 2008. Simultaneous inference in general parametric models. Biometric Journal 50: 346-363.

Based on statistically significant adverse effects on adult worker honeybee mortality and foraging activity, the no-observed adverse effect level (NOAEL) across the various measurement endpoints for afidopyropen is <50 g a.i./ha under the conditions tested for this treatment.

Reviewer's Comments:
The reviewer's overall results and conclusions agreed with those of the study author, and in spite of some differences regarding approaches towards statistically analyzing the study data, the reviewer and the study author agreed on the significance of treatment responses for particular endpoints. The study author did not statistically analyze colony strength or condition data, so comparisons between the reviewer's and study author's conclusions for these endpoints are not possible. The reviewer's and study author's treatment mean calculations differ slightly for the pre-application and exposure phases of the study. This discrepancy may be due to the study author's coding of data collected on the day of applications, or differences in incorporation of the 0 DAT data into treatment mean calculations. The reviewer included all data from the day of applications that were collected prior to applications (*i.e.* "0ba") in the pre-application phase dataset, and all data from the day of applications that were collected after applications (*i.e.* "0aa") in the exposure phase dataset; it's not entirely clear from the study report whether the study author employed the same distinctions.

In terms of statistical approaches, the study author claimed in the study report that data were tested to see whether they met assumptions of parametric tests, and the statistical tests used by the author are all parametric tests. However, the reviewer's analysis indicated that several of the datasets analyzed in this manner by the study author did not meet assumptions for parametric tests, and should have been analyzed using non-parametric tests. This difference in statistical approaches may explain why the reviewer's analysis of foraging activity data found that there was a significant ($p<0.05$) reduction in foraging activity in afidopyropen-treated colonies during the exposure phase of the study, but that the study author did not make this same statistical conclusion.

According to the study author, applications to the 2.2-ha control plot took place between 9:15 to 9:35 hrs (20 min), and application to the 2.1-ha afidopyropen plot took place from 10:23 to 10:43 hrs (20 min); the afidopyropen plot is roughly 5% smaller than the negative control. Seven colonies were placed in each of the plots; however, since the plot itself is the area treated, the plot represents the experimental unit rather than the seven colonies placed within the treated plot. Therefore, technically, the individual colonies within the plot are pseudo-replicates.

The study author noted that during the pre-application phase of the study there was 4-5 mm of rainfall (and 60-70% cloud cover) 3 days before application. Additionally, during the exposure phase of the study mean daily temperatures were 15.3-29.6 °C, and there was substantial rainfall at 6 (10-14 mm), 8 (7-9 mm) and 9 DATs (3 mm). OECD Guidance Document No. 75 notes that daytime temperatures below 15 °C may inhibit honeybee foraging activity. While these adverse environmental conditions would have theoretically affected all treatment groups equally, nevertheless they result in some uncertainty regarding the degree of foraging activity of colonies at the time of applications, and during the exposure phase of the study.

Reviewer's Conclusions:
The full-field study was initiated in June 2015 with the afidopyropen formulated end-use product BAS 440 00 I (VERSYS™, 9.8% active ingredient) applied at full bloom during active bee foraging (*i.e.*,

daytime). Bee colonies in the negative control and 50 g a.i./ha BAS 440 00 I (0.04 lbs a.i./A) treatments were assessed at multiple time points. Treatment rates were not confirmed analytically; however, afidopyropen residue samples were collected from relevant phacelia matrices to verify applications. Colonies were exposed to treated fields for nine days (0 – 9 DAT), and then moved to a remote monitoring location for 34 days (10 – 43 DATs).

There were no statistically significant differences in adult worker bee mortality between the negative control and afidopyropen-treated colonies during the pre-application or monitoring phases of the study; during the exposure phase of the study, mean adult worker bee mortality was significantly ($p<0.05$) different (*i.e*., 33% higher) in colonies treated with afidopyropen relative to negative control colonies. Worker bee mortality was particularly high in afidopyropen treated colonies on 0 DAT after applications were made and on the day following applications, by 2 DAT worker bee mortality in afidopyropen colonies was similar to that in negative control colonies. No dead pupae were found in negative control or afidopyropen colonies during the pre-application, exposure and monitoring phases. There were no statistically significant ($p <0.05$) differences in foraging activity between afidopyropen-treated colonies and the negative control during the pre-application phase of the study; during the exposure phase of the study, relative to negative control colonies mean foraging activity was significantly ($p <0.05$) different (*i.e*., 11% lower) in afidopyropen-treated colonies. Foraging activity in afidopyropen colonies was particularly reduced on 0 DAT following applications, and by 4 DATs foraging activity in the afidopyropen and negative control colonies was similar.

Sublethal behavioral effects after afidopyropen-treatment on the day of application were noted, wherein approximately 200 bees were reported as falling from flowers during foraging or inactivity; however, no additional behavioral effects in honeybees in the afidopyropen colonies were noted for the remainder of the assessment period (i.e., 1-43 DATs).

There were no statistically significant ($p <0.05$) differences in mean colony weight between afidopyropen-treated colonies and the negative control at either the beginning of the study (*i.e*., -1 DAT) or the conclusion of the study (*i.e*., 43 DAT). The mean number of adult worker bees in afidopyropen-treated colonies was comparable to that in negative control colonies throughout the study except for 10 DAT; the mean number of worker bees in afidopyropen-treated colonies 10 DATs was significantly ($p<0.05$) different (*i.e.*, 11% higher, respectively) than the mean number of worker bees in the negative control colonies. The mean number of brood (eggs, larvae and pupae) and food (nectar and pollen) cells in afidopyropen-treated colonies were comparable to that in negative control colonies throughout the study.

There were inclement weather conditions during the pre-application period, *i.e.*, 4-5 mm of rainfall (and 60-70% cloud cover) 3 days before application. There was also substantial rainfall (> 5 mm) periodically throughout the exposure and monitoring phases of the study. Additionally, because nominal treatment levels of afidopyropen were not verified analytically and use of a reference toxicant was not suitable, there is some uncertainty regarding actual exposure levels. Measured residues of afidopyropen and its transformation product M4401007 indicate that colonies were exposed to the afidopyropen treatment.

The study was generally consistent with OCSPP Guideline 850.3040, and indicates that honey bee colonies in a field of phacelia at full bloom treated with formulated afidopyropen at 50 g a.i./ha (0.04 lbs a.i./A) during active bee flight (*i.e*., in the daytime) exhibited significant adverse effects on adult worker

bee mortality (*i.e.*, 33% higher) and foraging activity (*i.e.*, 11% lower). Adverse effects on worker bee mortality and foraging activity occurred primarily on the day of applications (0 DAT) and on 1 DAT, and by the conclusion of the study (43 DAT) afidopyropen-treated colonies appeared to be similar (with respect to colony strength, brood strength and food stores) to negative control colonies. Based on this study and the noted transient, but statistically significant effects, the NOAEL is <50 g a.i./ha for applications during active bee flight.

EPA Classification: Supplemental (should only be used qualitatively)
PMRA Classification: Reliable with restrictions

APPENDIX I. Output of Statistics Verified by the Reviewer

Adult Honeybee Mortality (no. dead bees/colony/d)

```
Call: lm(formula = value ~ trtmnt + phase, data = z)

Residuals:
   Min     1Q Median     3Q    Max
-7.349 -1.978 -0.602  1.398 53.487

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   6.1366     0.3418  17.956  < 2e-16 ***
trtmnttest    0.3761     0.2927   1.285  0.19920
phasemon     -3.5348     0.3553  -9.948  < 2e-16 ***
phasepre      1.8360     0.5981   3.070  0.00223 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 3.833 on 682 degrees of freedom
Multiple R-squared:  0.2012,     Adjusted R-squared:  0.1976
F-statistic: 57.24 on 3 and 682 DF,  p-value: < 2.2e-16

Shapiro-Wilk normality test
W = 0.73884, p-value < 2.2e-16

Bartlett test of homogeneity of variances
Bartlett's K-squared = 43.861, df = 1, p-value = 3.526e-11

Bartlett test of homogeneity of variances
Bartlett's K-squared = 211.67, df = 2, p-value < 2.2e-16

Pre-application Phase
Wilcoxon rank sum test with continuity correction
W = 363.5, p-value = 0.6452

Exposure Phase
Wilcoxon rank sum test with continuity correction
W = 2402.5, p-value = 0.04142

Monitoring Phase
Wilcoxon rank sum test with continuity correction
W = 29936, p-value = 0.2759
```

Foraging Activity (bees/m²/d)

```
Call: lm(formula = value ~ trtmnt + phase, data = z)

Residuals:
    Min      1Q  Median      3Q     Max
-5.2961 -1.1723 -0.0144  1.5461  3.5461

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  10.0144     0.2078  48.192  < 2e-16 ***
trtmnttest   -0.8421     0.2761  -3.051  0.00262 **
phasepre     -1.7183     0.3386  -5.075 9.29e-07 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.903 on 187 degrees of freedom
  (647 observations deleted due to missingness)
Multiple R-squared:  0.1579,     Adjusted R-squared:  0.1489
```

```
F-statistic: 17.53 on 2 and 187 DF,  p-value: 1.05e-07

Shapiro-Wilk normality test
W = 0.94642, p-value = 1.522e-06

Bartlett test of homogeneity of variances
Bartlett's K-squared = 0.79931, df = 1, p-value = 0.3713

Bartlett test of homogeneity of variances
Bartlett's K-squared = 1.6744, df = 1, p-value = 0.1957

Pre-application Phase
Wilcoxon rank sum test with continuity correction
W = 192, p-value = 0.8369

Exposure Phase
Wilcoxon rank sum test with continuity correction
W = 3695, p-value = 0.0007181
```

Colony Weight (kg)

```
Call: lm(formula = value ~ trtmnt + dat, data = z)

Residuals:
    Min      1Q  Median      3Q     Max
-3.7429 -2.0429 -0.1357  1.6071  4.4571

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 26.61201    0.74754  35.599  < 2e-16 ***
trtmnttest   1.40000    0.87626   1.598  0.12268
dat          0.06916    0.01992   3.473  0.00189 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 2.318 on 25 degrees of freedom
Multiple R-squared:  0.3689,     Adjusted R-squared:  0.3184
F-statistic: 7.306 on 2 and 25 DF,  p-value: 0.003173

Shapiro-Wilk normality test
W = 0.9701, p-value = 0.5832

Bartlett test of homogeneity of variances
Bartlett's K-squared = 0.22255, df = 1, p-value = 0.6371

Bartlett test of homogeneity of variances
Bartlett's K-squared = 0.67695, df = 1, p-value = 0.4106

Pre-application phase (-1 DAT)
Welch Two Sample t-test
t = -1.1902, df = 11.723, p-value = 0.2575

Monitoring phase (43 DAT)
Welch Two Sample t-test
t = -1.0223, df = 11.962, p-value = 0.3269
```

Colony Strength (no. adult bees/colony/d)

```
Call: lm(formula = value ~ trtmnt + dat, data = z)

Residuals:
    Min      1Q  Median      3Q     Max
-2832.0 -1285.2  -250.6   999.0  4916.3
```

```
Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) 14433.5328   339.0851  42.566   <2e-16 ***
trtmnttest    324.1429   372.5642   0.870    0.387
dat            -0.2155    11.9712  -0.018    0.986
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1707 on 81 degrees of freedom
Multiple R-squared:  0.009263,    Adjusted R-squared:  -0.0152
F-statistic: 0.3786 on 2 and 81 DF,  p-value: 0.686

Shapiro-Wilk normality test
W = 0.94056, p-value = 0.0007431

Bartlett test of homogeneity of variances
Bartlett's K-squared = 0.231, df = 1, p-value = 0.6308

Bartlett test of homogeneity of variances
Bartlett's K-squared = 24.273, df = 5, p-value = 0.0001924

-1 DAT
Wilcoxon rank sum test
W = 30, p-value = 0.535

3 DAT
Wilcoxon rank sum test with continuity correction
W = 29.5, p-value = 0.5644

10 DAT
Wilcoxon rank sum test with continuity correction
W = 8, p-value = 0.04047

21 DAT
Wilcoxon rank sum test with continuity correction
W = 18, p-value = 0.4428

31 DAT
Wilcoxon rank sum test with continuity correction
W = 18, p-value = 0.4418

43 DAT
Wilcoxon rank sum test with continuity correction
W = 27.5, p-value = 0.7489

--------------------------------------------------------------------------------
Colony Condition - Brood (no. cells/colony/d as brood)
Call: lm(formula = value ~ trtmnt + dat, data = z)

Residuals:
    Min      1Q  Median      3Q     Max
-2633.4 -1199.0  -588.1  1094.0  4801.7

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 3042.791    189.545  16.053   <2e-16 ***
trtmnttest    76.916    208.260   0.369    0.712
dat           -2.966      6.692  -0.443    0.658
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1653 on 249 degrees of freedom
Multiple R-squared:  0.001335,    Adjusted R-squared:  -0.006686
F-statistic: 0.1664 on 2 and 249 DF,  p-value: 0.8468
```

```
Shapiro-Wilk normality test
W = 0.88546, p-value = 7.353e-13

Bartlett test of homogeneity of variances
Bartlett's K-squared = 0.050529, df = 1, p-value = 0.8221

Bartlett test of homogeneity of variances
Bartlett's K-squared = 11.279, df = 5, p-value = 0.04613

-1 DAT
Wilcoxon rank sum test with continuity correction
W = 222.5, p-value = 0.9699

3 DAT
Wilcoxon rank sum test with continuity correction
W = 227, p-value = 0.8799

10 DAT
Wilcoxon rank sum test with continuity correction
W = 202.5, p-value = 0.6594

21 DAT
Wilcoxon rank sum test with continuity correction
W = 201.5, p-value = 0.6414

31 DAT
Wilcoxon rank sum test with continuity correction
W = 198, p-value = 0.5797

43 DAT
Wilcoxon rank sum test with continuity correction
W = 257.5, p-value = 0.3581

--------------------------------------------------------------------------------
Colony Condition - Food (no. cells/colony/d as food)
Call: lm(formula = value ~ trtmnt + dat, data = z)

Residuals:
   Min     1Q Median     3Q    Max
 -6199  -4438  -2018   4865  10297

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  5925.51     679.22   8.724  2.8e-15 ***
trtmnttest   -445.54     746.28  -0.597    0.551
dat            27.30      23.98   1.139    0.256
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 4836 on 165 degrees of freedom
Multiple R-squared:  0.009919,   Adjusted R-squared:  -0.002082
F-statistic: 0.8265 on 2 and 165 DF,  p-value: 0.4394

Shapiro-Wilk normality test
W = 0.8716, p-value = 8.369e-11

Bartlett test of homogeneity of variances
Bartlett's K-squared = 0.00058616, df = 1, p-value = 0.9807

Bartlett test of homogeneity of variances
Bartlett's K-squared = 13.743, df = 5, p-value = 0.01733

-1 DAT
Wilcoxon rank sum test with continuity correction
W = 111, p-value = 0.5656
```

3 DAT
Wilcoxon rank sum test with continuity correction
W = 93, p-value = 0.8361

10 DAT
Wilcoxon rank sum test with continuity correction
W = 102, p-value = 0.8722

21 DAT
Wilcoxon rank sum test with continuity correction
W = 120, p-value = 0.3229

31 DAT
Wilcoxon rank sum test with continuity correction
W = 116, p-value = 0.4211

43 DAT
Wilcoxon rank sum test with continuity correction
W = 109.5, p-value = 0.6131